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Kinetic of Adjustment of Enzyme Catalytic Concentrations in the Extracellular Space of the Man, the Dog and the Rat

Approach to a Quantitative Diagnostic Enzymology, V. Communication

By *J. Lindena, F. Diederichs, H. Wittenberg* and *I. Trautschold†*

Abteilung Klinische Biochemie, Medizinische Hochschule Hannover

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In memoriam Professor Dr. Dr. Ivar Trautschold († 31. 1. 1984)

Summary: The high degree of constancy of enzyme catalytic activity in the plasma of a given individual is regulated by a complex system of flux equilibria consisting of eight basic processes. Some of these processes are of primarily theoretic importance. Enzymes from all tissues of the body, including the liver, are released via a continuous physiological process into the interstitial space and get into the intravascular space by way of lymphatic transport. The release of enzymes from tissues directly into the intravascular space is of secondary importance as is the exchange of enzyme molecules across capillary membranes from the intravascular to the interstitial space and vice versa. In contrast, enzymes from circulating blood cells are transported directly into the intravascular space. Enzymes are removed from the intravascular space at rates which vary greatly between both enzymes and species. In a review of the literature, half-lives of diagnostically important enzymes in plasma of man, dogs and rats were given and the striking differences in the results for a given enzyme are discussed from a methodological point of view. In a mathematical analysis, data for lymphatic transport of enzymes from dogs and rats (*Lindena* et al. (1986) this J. *24*, 19–33) and of enzyme efflux from in vivo ageing erythrocytes (*Lindena* et al. (1986) this J. *24*, 49–59) into the plasma are related to the elimination rate constants of enzymes from the plasma. The contribution of lymphatically transported enzymes to the basal catalytic activity in plasma (*Lindena & Trautschold* (1986) this J. *24*, 11–18) amounts to 55–80% for lactate dehydrogenase and malate dehydrogenase, 80–90% for adenylate kinase and phosphohexose isomerase, 90–95% for aspartate aminotransferase and aldolase and 99% for creatine kinase. A model of Ca^{2+}-mediated vesicular transport of enzymes out of ageing erythrocytes is proposed. The importance of lymphatically transported enzymes to total plasma catalytic activity in dogs and rats argues for a similar contribution of lymph transport in man.

Die Kinetik der Einstellung der katalytischen Konzentration von Enzymen im Extrazellularraum bei Mensch, Hund und Ratte
Versuch der Begründung einer quantitativen Diagnostischen Enzymologie, V.

Zusammenfassung: Die hohe intraindividuelle Konstanz von katalytischen Enzymaktivitäten im Plasma wird durch ein komplexes System von Fließgewichten bestehend aus acht Grundprozessen aufrechterhalten, von denen einige aber mehr von theoretischer Bedeutung sind. Aus allen Geweben des Körpers, einschließlich der Leber, gelangen Enzyme – freigesetzt in einem kontinuierlichen physiologischen Vorgang – in den interstitiellen Raum und von da über lymphatischen Transport in den Intravasalraum. Die Freisetzung von Enzymen aus Geweben direkt in den Intravasalraum als auch der Austausch von Enzymen vom Intravasalraum in den interstitiellen Raum über die Kapillarmembran und vice versa ist von untergeordneter Bedeutung.

Enzyme aus zirkulierenden Blutzellen dagegen gelangen direkt in den Intravasalraum. Aus dem Intravasalraum werden Enzyme mit konstanten Raten eliminiert, die sich sowohl für die einzelnen Enzyme als auch die Spezies deutlich unterscheiden. Eine Literaturübersicht der Halbwertszeiten von diagnostisch bedeutsamen Enzymen im Plasma für Mensch, Hund und Ratte wurde erstellt und die deutlichen Unterschiede in den Ergebnissen für das gleiche Enzym werden unter methodischen Gesichtspunkten diskutiert. Eine Berechnung für Hund und Ratte stellt die Ergebnisse des lymphatischen Transports von Enzymen (*Lindena* et al. (1986) this J. *24*, 19–33) und des Enzymefflux von in vivo alternden Erythrocyten (*Lindena* et al. (1986) this J. *24*, 49–59) in das Plasma der Eliminationskonstante von Enzymen aus dem Plasma gegenüber. Der Anteil des lymphatischen Transports am Gesamtfluß zur Einstellung der basalen katalytischen Aktivität im Plasma (*Lindena & Trautschold* (1986) this J. *24*, 11–18) beträgt etwa 55–80% für Lactatdehydrogenase und Malatdehydrogenase, 80–90% für Adenylatkinase und Phosphohexoseisomerase, 90–95% für Aspartataminotransferase und Aldolase und 99% für Kreatinkinase. Es wird ein Modell vorgeschlagen, das den Austritt von Enzymen aus alternden Erythrocyten über eine Ca^{2+}-vermittelte Vesikulation von Erythrocytenmaterial erklärt. Die Bedeutung des lymphatischen Transports von Enzymen für die Einstellung der basalen katalytischen Aktivität im Plasma bei Hund und Ratte läßt auf eine ähnliche Bedeutung des Lymphtransportes für den Menschen schließen.

Introduction

The catalytic activity of enzymes in plasma is regulated by a complex system of flux equilibria which most likely consists of the processes shown diagramatically in figure 1.

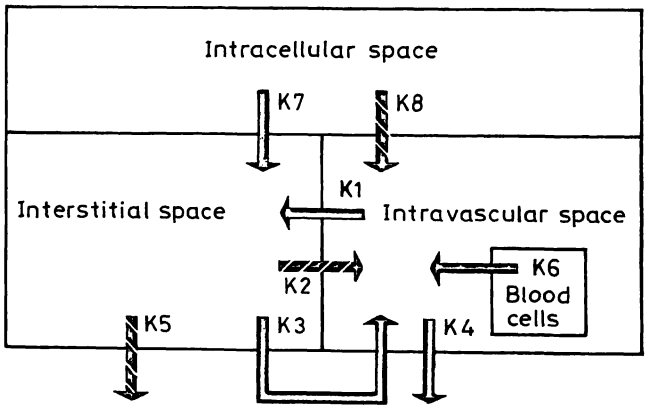

Fig. 1. Diagramatic illustration of processes responsible for the adjustment of enzyme catalytic activities in blood plasma. For further details see text. Modified from *Friedel* et al. (1).

1. The release of enzymes either from tissue cells into the interstitial and/or intravascular space, characterized by the rate constants K 7 and K 8, respectively, or the release of enzymes from blood cells into the intravascular space, characterized by the rate constant K 6.
2. The exchange rate of enzyme molecules across capillary membranes from the intravascular to the interstitial and from the interstitial to the intravascular space, characterized by the rate constants K 1 and K 2, which are measures of the capillary permeability.
3. The elimination of enzyme molecules either from the intravascular or the interstitial space, characterized by the rate constants K 4 and K 5.
4. The transport of enzyme molecules from the interstitial into the intravascular space via the lymph. The constant K 3, characterizing this process, is a measure of the lymphatic transport.

This rather confusing systems of flux equilibria consisting of eight basic processes can be reduced to only five processes. This became evident from our investigation on thoracic duct, hepatic and intestinal lymph (2). We have shown that enzymes released from the liver and other parenchymal tissues reach the intravascular space primarily via the lymphatics. Direct contributions of enzymes from the intracellular to the intravascular space (K 8), as well as exchanges across capillary membranes from the interstitial to the intravascular space (K 2), are of secondary importance. In addition, the elimination of enzymes from the interstitial space (K 5) is negligible (3, 4). Rate constants of significant importance are represented by solid lines, rate constants of minor importance by dashed lines. The ground work for quantitative interspecies comparisons of enzyme kinetics in the extracellular space has been laid in previous publications. Catalytic activity in plasma, the amount of lymphatically transported catalytic activity, and the efflux of catalytic activity from ageing erythrocytes have already been estimated for a number of species in these studies (2, 5, 6). Other fundamentals essential in considering overall enzyme flux, such as exchange rates of enzymes from the intravascular to the interstitial space, and plasma elimination rates, were obtained from the liteterature and reviewed. Our aim was to determine the precise sources of plasma en-

zyme catalytic activity and their degree of relative importance. Under steady-state conditions, the catalytic activity of diagnostically relevant enzymes has been found to be highly constant for any given individual in both man (7, 8) and rats (9). The basal catalytic activity is the sum of contributing and eliminating rate constants, which are considered to be of almost equal magnitude, and thereby result in a steady-state.

Components which should contribute to the basal plasma catalytic activity have been quantitied in several animal species. These include lymphatic transport (2) and the contribution of ageing erythrocytes (6). The sum of these essential contributing rate constants should be equal to the elimination rate constant. With regard to man, data for lymphatic transport of enzymes is not obtainable and complete quantitative calculations are therefore restricted to animals such as the dog and the rat.

Results of these animal studies can be related to the situation in human physiology.

Materials and Methods

Our calculations are based on the following assumptions:

1. Enzymes released from tissues in a constant physiological process enter the blood only by lymphatic transport.
2. Thoracic duct lymph contains 60% of lymphatically transported enzymes. The right duct, the subclavian and the cervical ducts account for the other 40%. These other ducts have the same enzyme patterns as the thoracic duct.
3. Enzymes are released from ageing erythrocytes in catalytically active form.
4. The transport rate of enzyme molecules across the capillary membrane from the interstitial to the intravascular space (K 2) is negligible.
5. The amount of enzymes released from tissues (K 7) is represented by the lymphatic transport of enzymes (K 3).
6. The elimination of enzyme molecules from the interstitial space (K 5) is negligible.
7. Flux of enzyme molecules from the intravascular to the interstitial space (K 1) is included in the lymphatic transport of enzymes (K 3).

The validity of these assumptions will be discussed.

The following data were used for calculation:

Lymphatic transport of enzymes (K 3)

Table 2c from the II. communication of this series (2) listed the lymphatic transport of enzymes in the thoracic duct. Data are assumed to account for 60% of total body lymph enzyme transport and are extrapolated to 100%. The values for conscious dogs and for anaesthetized rats during passive motion were taken.

Release of enzymes from blood cells (K 6)

We have calculated the daily loss of catalytic activity of total body erythrocytes in man, dogs and rats in table 1 of the IV. communication of this series (6).

Normal catalytic activity in plasma

Values for man, dogs and rats were taken from table 1 of the I. communication (5).

Elimination constant (K 4)

Data for man, dogs and rats have been reviewed from the literature and are listed in table 1 of the present investigation.

Plasma volume

Man:
Mean body weight 67 kg; plasma volume 43.5 ml/kg body weight (79).

Dog:
Mean body weight 31 kg; plasma volume 50 ml/kg body weight (80).

Rat:
Mean body weight 260 g; plasma volume 36.8 ml/kg body weight (81).

Mathematical analysis of data

According to the diagrammatic illustration of figure 1, and based on the above mentioned assumptions the time dependent change of the intravascular enzyme catalytic activity concentration (U/l) depends on lymphatic transport (K 4), erythrocyte derived enzymes (K 6) and the elimination of enzymes from plasma (K 4).

$$\frac{dC}{dt} = K - K4 \cdot C \qquad \text{(Eq. 1)}$$

where K is the sum of the lymphatic influx of enzymes and of the efflux of enzymes from erythrocytes into the plasma, both of which are constants. K 4 is the elimination constant. Integration of equation 1 results in

$$C = \frac{K4\,C_0 - K}{K4} \cdot e^{-K4t} + \frac{K}{K4} \qquad \text{(Eq. 2)}$$

where C_0 is the plasma catalytic activity concentration after a bolus injection at $t = 0$. It follows that the steady-state catalytic activity concentration ($t \rightarrow \infty$) is given by:

$$\frac{K}{K4}$$

Results and Discussion

Elimination of enzymes from the intravascular space (K 4)

We should begin by discussing the confusing literature data for elimination rate constants of enzymes. These are reviewed in table 1. It is clear that these values often differ by more than a factor of 10, especially in man and to a lesser degree in dogs. Data from rats are rare.

Tab. 1. Half-lives (h) of diagnostically important enzymes in plasma of man, dogs and rats, as reported in the literature. Mean values or ranges are given.

Enzyme	Man		Dog		Rat	
	Half-life	References	Half-life	References	Half-life	References
Lactate dehydrogenase	46.8; 73.0; 23.3–91.2; 40; 114	10; 11; 12; 13; 14	1.6	57		
Isoenzyme 1 (H4)	113; 48; 59; 72	15; 16; 17; 18	3.3	3	5.3	75
Isoenzyme 2 (H3 M1)					4.1	75
Isoenzyme 3 (H2 M2)					1.5	75
Isoenzyme 4 (H1 M3)					0.84	75
Isoenzyme 5 (M4)	10; 7.4; 8; 14.4	15; 16; 17; 19	0.8; 1.8; 0.5–0.7	3; 58; 59	0.46–0.54; 0.42; 0.9; 0.5; 0.6	76; 75; 77; 78; 3
α-Hydroxybutyrate dehydrogenase	45.9; 46.2; 44.4; 15.9–80.6; 116; 57.8	10; 20; 21; 12; 14; 22	2.2; 1.9	58; 20		
Malate dehydrogenase	16; 12; 18	15; 13; 23			0.58	77
Isoenzyme, mitochondrial	18.4	23	3.3; 28	3; 60		
Isoenzyme, cytosolic			2.6; 14	3; 60		
Glutamate dehydrogenase	18; 16	15; 24	7.9; 16	58; 60	4.0	77
γ-Glutamyl transferase	98.4	25				
Aspartate aminotransferase	17; 11.1; 22; 4.0–45.6; 4.7; 40 12.5; 24; 20; 8.1	15; 10; 11; 12; 13; 14 26; 27; 28; 22	3.5; 4.4; 3.3	57; 58; 61	2.3	77
Isoenzyme, mitochondrial	6	16	6.1; 6.0; 6.8	3; 60; 62	1.2–2.3	62
Isoenzyme, cytosolic	12–17	16	3.2; 12; 11.8	3; 60; 63		
Alanine aminotransferase	47; 32; 41; 52; 43	15; 11; 24; 28; 29	31.5; 2.5; 60.9	57; 58; 64	4.4	77
Creatine kinase	15; 9.6; 3.5; 12.4; 4.7–35.2; 4.5; 24; 7.0 18; 20; 5.0; 23.9–25.6; 19.3; 5.8–19.3; 12.5 40–53; 10.6; 15.5; 10.5; 3.6; 11.6	15; 10; 20; 21; 12; 13; 14; 26 27; 28; 30; 31; 32; 33; 34 35; 36; 37; 38; 22; 39	4.6–16.2; 1.9; 0,58	65; 20; 4	0.55	77
Isoenzyme MB	7.0; 9.6; 17.8–21; 16.5; 5.0–9.6; 18.7; 9.5 9.4; 9.4; 5.8; 12.5; 6.8; 6.1; 3.2	27; 40; 31; 32; 33; 41; 42 43; 44; 45; 37; 38; 39; 30	3.8–8.1; 1.3	65; 66		
Isoenzyme MM	37.5; 13.4; 4.4	41; 43; 30	1.7–3.0; 4.2–16; 1.2–2.6 2.5; 1.8; 1.9; 1.9; 2.4 2.4; 1.1; 2.3–6.4	67; 65; 68 69; 66; 61; 57; 70 71; 72; 4		
Isoenzyme BB	3.0	46	0.69	66		
Adenylate kinase	2.6	47			0.85	77
Cholinesterase	81.6; 240; 81.6; 288	48; 49; 50; 51				
Alkaline phosphatase						
Isoenzyme, biliary	232; ~144	25; 52	66; ~144	73; 52		
Isoenzyme, placental	~144; 166	52; 53	0.09	73		
Isoenzyme, skeletal	26.9–51.6	54				
Isoenzyme, intestinal			0.1	73		
Isoenzyme, renal			0.06	73		

Tab. 1. Continued.

Enzyme	Man		Dog		Rat	
	Half-life	References	Half-life	References	Half-life	References
Acid phosphatase						
Isoenzyme, prostatic	~4; 1.2	52; 55				
α-Amylase	3–6; ~4	56; 52				
Isoenzyme, pancreatic	9.3–17.7	74	1.3–4.0	52	1.3–4; 0.34	52; 74
Amino acid arylamidase	209	25				
Aldolase	21	15			4.4	77
Phosphohexose isomerase	4.1; 4.2–70	10; 12				

It is unlikely that these large differences result from differences in the basic mechanisms of enzyme elimination (1, 2, 72, 75, 76, 78, 82, 83). It is much more likely, rather that they are the result of differences in methodology.

Most data for man originate from clinical observations of exponential fits for plasma enzyme levels after myocardial infarction, liver failures and other disorders. These clinical studies are based on the assumption that the particular event had an end point with respect to the pathological enzyme release and the increased inflow of enzymes into the intravascular compartment. Such assumptions are questionable, however. Enzymes are released for up to 96 h after myocardial infarction. Therefore, estimation of the elimination constant from the rate of plasma disappearance 36 or 48 h after the infarct results in large underestimations (20). In addition, release of enzymes from secondarily affected organs might change the typical disappearance pattern of a particular enzyme. Resupply of the vascular pool from the extravascular space by delayed lymphatic enzyme transport into the blood could further slow the disappearance rate. Results obtained by such studies on apparent half-life of enzymes in plasma are nevertheless most helpful. The knowledge of a true and exact elimination rate might, in fact, be irrelevant to a physician trying to make a diagnosis. The knowledge of apparent disappearence rates obtained from typical uncomplicated cases is of considerable value because deviations therefrom can sometimes signal the development of complications or other pathological conditions (1).

A more reliable or true elimination rate as needed for our quantitative approach can be measured if an enzyme level can be acutely elevated in the intravascular compartment and if it can be subsequently ensured that inflow has definitely ceased. Such a condition can be achieved only by intravenous injection of enzymes, which is mostly done in animal studies. Only a few injection studies have been performed in humans (30, 49, 50, 53, 84).

These animal studies have led to a better understanding of the complex mechanism involved in estimating elimination constants and more elaborate mathematical procedures have been introduced which are based upon description of plasma curves as a sum of exponentials, each exponential corresponding to a separate compartment. It has been shown that the elimination, for instance, of creatine kinase is 3 to 4-times faster in man and animals than previously reported (4, 20). There remains, however, considerable controversial discussion between the few groups involved with this problem with regard to specific enzyme elimination constants (4, 20, 61, 70, 85).

Some of the assumptions used for the calculations in table 1 are of a dubious nature. It also cannot be decided in every case whether the true or the apparent half-life has been determined. As a compromise, therefore, we have related our data on lymphatic transport rate and erythrocyte derived enzyme efflux under steady-state conditions (K) to both the fastest and the slowest elimination constants (K 4).

The uncertainties inherent in the literature data may explain some of the obvious discrepancies in our results (tab. 2, tab. 3). The lymphatic transport rate for malate dehydrogenase in dogs, for aspartate aminotransferase in dogs and rats, and for alanine aminotransferase in rats exceeds the value of the elimination rate. For both aminotransferases in the rat and for malate dehydrogenase in dogs, it is obvious that the apparent half-live has been determined (60, 77). It follows, that the respective values for K 4 in table 2 should be higher and the predicted values

$$\frac{K}{K4}$$

in table 3 should be lower.

Tab. 2. Comparison of eliminating and contributing rate constants to normal catalytic activity in plasma of man, dogs and rats.
K4 is the elimination of enzymes from the intravascular space computed from the normal catalytic activity in plasma (5), the plasma volume and from mean values or the fastest and slowest half-lives listed in tab. 1.
K3 is the rate constant of lymphatically transported enzymes (2).
K6 is the erythrocyte-derived enzyme flux (6).
All rate constants are given in U/h; n. d. not determined; – not known.

	Man		Dog			Rat		
	K4	K6	K4	K3	K6	K4	K3	K6
Lactate dehydrogenase	2.1 –10.0	2.1	17.1	11.8	2.5	0.30–0.59	0.17	0.14
Malate dehydrogenase	5.2 – 7.8	4.1	3.3 –17.8	23.0	10.6	0.29	0.21	0.07
Aspartate aminotransferase	0.32– 3.6	0.95	2.5 – 3.3	6.5	0.77	0.087	0.098	0.004
Alanine aminotransferase	0.43– 0.70	n. d.	0.32– 7.9	2.0	n. d.	0.035	0.098	n. d.
Pyruvate kinase	–	1.3	–	n. d.	2.3	0.067	n. d.	0.019
Creatine kinase	2.0 –29.9	0.23	1.5 –41.3	29.3	1.0	0.97	0.38	0.004
Adenylate kinase	–	14.8	–	n. d.	8.9	0.22	0.10	0.031
Aldolase	0.11	0.23	n. d.	5.5	0.47	0.023	0.021	0.004
Phosphohexose isomerase	0.73–12.5	2.7	n. d.	21.8	6.5	n. d.	0.22	0.018

Tab. 3. Comparison of normal catalytic activity concentration (U/l) in plasma of dogs and rats vs. the calculated catalytic activity concentration.
Normal catalytic activity is taken from the I. communication of this series (5).
$\frac{K}{K4}$ (U/l) is the calculated catalytic activity which is based on values of lymphatic influx of enzymes (2) and of the influx into the plasma from ageing erythrocytes (6) divided by elimination constant. See equation 2 under Materials and Methods; – elimination constant not known.

	Dog		Rat	
	$\frac{K}{K4}$	Normal values	$\frac{K}{K4}$	Normal values
Lactate dehydrogenase	21.2	25.4	21.3–41.7	44.1
Malate dehydrogenase	81.0–436	43.0	24.6	25.7
Aspartate aminotransferase	22.1– 29.5	10.1	58.1	29.9
Alanine aminotransferase	4.7–113	18.3	64.8	23.4
Creatine kinase	16.3–457	22.2	31.7	79.8
Adenylate kinase	–	20.1	17.0	28.6
Aldolase	–	7.4	25.7	15.5

Lymphatic transport of enzymes (K 3)

The significance of lymphatic transport of enzymes from tissues into the blood for the level of enzymes in plasma has been argued by *Friedel* et al. (86), our group (87–90) and others.

Our quantitative approach to an explanation of the exercise-induced changes in catalytic activities in plasma by the lymphatic influx of enzymes from the interstitial resting enzyme pool in muscle was convincing (91). In a previous communication of this series (2) we presented data on thoracic duct enzyme transport of dogs, rabbits, rats and mice. These bore a greater resemblance to physiological conditions than did previous studies. It was concluded that the thoracic duct flow and enzyme content should account for 50–70% of total body lymph flow and lymph enzyme transport.

For quantitative considerations the lymph enzyme transport values are therefore extrapolated from 60% to 100%.

It also became evident from this and the above mentioned investigations that neither a release of enzymes from tissue cells directly into the intravascular space, (K 8 in fig. 1) nor the exchange rate of enzyme molecules across capillary membranes from the interstitial to the intravascular space (K 2) are of any significant importance. The rather complex system of eight rate constants could therefore be reduced to only four constants, assuming that the amount of enzymes released from tissues (K 7) is represented by lymphatic transport of enzymes (K 3).

The transport rate of enzyme molecules across capillary membranes from the intravascular to the interstitial space (K 1) is not separately considered in our computations because the K 1-dependent flux recirculates via lymphatic transport (K 3); this will be discussed later. Table 2 relates the rate of enzyme entry into the plasma, characterized by lymphatically transported enzymes (K 3) and by erythrocyte-derived enzyme flux (K 6) to the rate of enzyme elimination from the plasma space (K 4). For man only the rate constants K 6 and K 4 can be related.

It is demonstrated that lymph enzyme transport from the tissues is the predominant source of plasma catalytic activity of diagnostically important cell enzymes. In every case the lymphatic enzyme transport exceeded the erythrocyte-derived contribution. Some discrepancies seen for some enzymes, where the lymphatic transport rate alone was higher than the elimination rate, have already been attributed above to methodological difficulties in the estimation of the true elimination rate constant and may also be due to different enzyme test conditions in these studies.

The general agreement between data for two different species (dogs and rats) seems to justify a similar role for enzyme lymph transport in humans.

The transport rate of enzyme molecules from the intravascular to the interstitial space (K 1) and extravascular enzyme distribution space

There is considerable uncertainty about these parameters at the present time. Certain misleading concepts and assumptions have been established over the years because the significance of enzymes in lymph has been neglected. It is assumed that the process of exchange of enzyme molecules across capillary membranes from the intravascular to the interstitial space occurs by diffusion (10, 12).

Diffusion, however, as is well known, is proportional to the differences in concentration between the intravascular and extravascular compartment. The permeability constant for this flux has been estimated to be between 0.01 to 0.13 litre per h in man and dogs (4, 12, 20, 22, 61, 92).

However, the fact that most enzymes are found in much higher catalytic activities in lymph than in plasma (2) has not been taken into account. Calculations, based on our data, of the enzyme concentration of creatine kinase in muscle tissue, lymph and plasma (2, 5, 89, 90) gave values of 2000 μmol/kg tissue, 50 nmol/l and 20 nmol/l, respectively. Diffusion certainly is not the mechanism of enzyme exchange from the intravascular to the extravascular space. It is more likely that enzymes accompany pressure-dependent bulk flow and associated protein leakage across the capillary, especially in regions where capillary permeability is high (e. g. liver).

We have proved that enzymes in hepatic lymph originate preferentially from hepatocytes (2). On the contrary, evidence has been presented that hepatic lymph derives the bulk of its export proteins such as albumin from the plasma (93–95), and that the lymph/plasma concentration ratio of different export proteins was inversely related to their molecular weight (93). Once enzymes have reached the circulating blood, they then should be handled in their passage from the hepatic sinusoids into liver lymph like any other protein. Even adenylate kinase, with a molecular weight of 21 000, has a lymph/plasma ratio of 2.4, which far exceeds the predicted ratio on a molecular weight dependent basis alone. Liver lymph enzyme transport accounts for only between about 10 and 20% of total lymphatic enzyme transport (2). Because the capillary permeability in other tissues is comparably much

lower, the significance of exchange of enzymes from the intravascular to the interstitial space is disregarded in our calculation. Its existence, however, should be kept in mind for the liver.

The flux of enzymes from the intravascular to the interstitial space should be identical for all enzymes (except adenylate kinase). There is much experimental evidence (96, 97) showing that the blood-lymph barrier does not discriminate in the passage of molecules if the molecular weight exceeds about 40 000. As the molecular weights of cell enzymes investigated to date are about between 70 000 and 160 000, only small differences in permeability are to be expected (20, 61). We have shown that adenylate kinase (molecular weight 20 000) in the thoracic duct, liver and intestinal lymph is primarily released from tissue cells into the interstitial space (2).

The extravascular distribution space has been estimated for several enzymes in man, dogs and rats and controversial results were obtained which not infrequently differed by a factor of 10 (tab. 4). These values were obtained by the same groups who contributed the elimination constant data discussed above. All cited studies were based upon bolus injections of enzyme preparations or elevation of catalytic activity after myocardial infarction. In this respect it may be true that only the plasma distribution compartment is important if the rate of exchange of enzymes between the compartments is much slower than the rate of plasma clearance (61).

Under steady-state conditions, however, a true extravascular distribution space for enzymes is obvious. The extracellular fluid volume consists of an interstitial fluid volume (13.0% per kg body weight in man) and the plasma volume (4.3% per kg body weight) (98).

An important property imparted to the interstitial matrix by mucopolysaccharides is the ability of the entangled gel reticulum to exclude solutes from a portion of the available intragel water space. The degree of exclusion is dependent on solute size and density of the mucopolysaccharide meshwork. A normal interstitium is capable of excluding albumin from 50% of its structure; γ-globulin is excluded from 70% of the matrix volume (99, 100).

It can be assumed, therefore, that the diagnostically important enzymes with molecular weights as mantioned above are distributed extravascularly in a volume that is almost twice the plasma volume.

Release of enzymes from bood cells (K 6)

To test the hypothesis that circulating erythrocytes make a significant contribution to the normal catalytic activity in plasma, it was assumed in the foregoing publication (6) as a working hypothesis, that the measured loss of catalytic activity in ageing erythrocytes is equivalent to the amount of the enzymes released in catalytically active form into plasma.

Tab. 4. Extravascular distribution space of enzymes expressed as percentage of intravascular (plasma) distribution space. The values indicated are means and/or ranges from the cited literature.

Enzyme	Species	Extravascular space (%)	Reference
Lactate dehydrogenase	man	30	12
	dog	300	3
	rat	116	3
α-Hydroxybutyrate dehydrogenase	man	40–238; 30; 81 (29–320)	22; 12; 20
Aspartate aminotransferase	man	40–238; 30	22; 12
	dog	65 (31–160)	61
Creatine kinase	man	40–238; 81 (29–320); 30; 18 (0–98)	22; 20; 12; 30
	dog	0; 74–134	67; 69
Isoenzyme MM	dog	65 (31–160); 130; 48 (35–79); 23 (5–41) −38– +47; 34 (20–66); 0	61; 71; 4; 70 69; 66; 33
Isoenzyme MB	dog	56 (20–106); 0	66; 67
Isoenzyme BB	dog	108 (50–191)	66
Phosphohexose isomerase	man	30	12

In dogs and rats the contribution from ageing erythrocytes accounts for 20–45% for lactate dehydrogenase and malate dehydrogenase, 10–20% for adenylate kinase and phosphohexose isomerase, 5–10% for aspartate aminotransferase and aldolase and only about 1% for creatine kinase. These relations roughly reflect the scaled intracellular erythrocyte catalytic activity pattern of these enzymes (101, 102). In man a similar order of magnitude can be calculated when one relates the rate constant from erythrocytes to that of the elimination constant. These observations make an erythrocyte-derived contribution to the basal plasma catalytic activity quite plausible. Convincing experimental evidence for such a contribution is lacking to date. There are, however, several further arguments which could support this hypothesis. *Diederichs* et al. (103) recently developed a theory of enzyme release which as a general rule may also hold true for erythrocytes. The following findings support this theory: In "old" erythrocytes ATP levels have been found to be greatly decreased, as the result of impaired glucose utilisation in the *Embden-Meyerhof* pathway (104–108). Low K^+ and high Na^+ and Ca^{2+} concentrations are also found in these cells (107, 109–113). In ageing erythrocytes decreased levels of total ATP-ase and Na^+/K^+ ATP-ase have been observed (107, 114, 115). Ca^{2+} ions leak into the cytoplasmic compartment and, at low ATP concentration, induce an outward directed blebbing and vesiculation of parts of the cell membrane by contractions of the erythrocyte cytoskeleton. Senescent erythrocytes have decreased cholesterol and phospholipid contents and decreased membrane surface (107). Release of spectrin-free vesicles from human erythrocytes during ATP depletion has been observed (116). This leads to a reduced mean corpuscular volume and an increased density (104, 107, 110, 117–119).

The actions of Ca^{2+} also may involve activation by Ca^{2+} of a K^+ channel in the membrane (120) and subsequent shrinking.

It must, however, be restated that there is no decisive proof whatsoever that the decline of catalytic activities in ageing erythrocytes is due to a loss by release. The reported contribution of enzyme efflux from in vivo ageing erythrocytes into the plasma in relation to lymphatic transport of enzymes is represented by maximal values.

Kinetic, isozymic and structural modifications of distinct enzymes (6) also point towards inactivation with age.

For enzymes of diagnostic importance the erythrocyte-derived enzymes, efflux is of no account.

The contribution of blood cells other than erythrocytes to the basal plasma catalytic activity is largely unknown. The small volume fraction of polymorphonuclear cells, e. g., in relation to red blood cells, is nearly totally compensated by their high intracellular catalytic activity (101, 102).

Several particulate and soluble stimuli induce not only these cells but also monocytes to release lysosomal enzymes with minimal associated leakage of cytoplasmic enzymes (120–124). In contrast to the well documented correlation between erythrocyte density, age, and diminishing enzyme content, the causes and importance of platelet heterogeneity remain controversial (125, 126).

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Joachim Lindena, DVM
Abteilung Klinische Biochemie
Medizinische Hochschule Hannover
Konstanty-Gutschow-Straße 8
D-3000 Hannover 61